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Genetic Divergence and mtDNA Lineages in Six Subspecies of Aoudad

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Abstract

DNA sequences from the mitochondrial cytochrome-*b* gene (*Cyt*b) were used to assess genetic divergence in native and introduced populations of aoudad (*Ammotragus lervia*) from northern Africa and the southwestern United States (California, New Mexico, and Texas), respectively. *Cyt*b sequences from 279 individuals, representing the six subspecies of aoudad (*A. l. angusi*, *A. l. blainei*, *A. l. fassini*, *A. l. lervia*, *A. l. ornata*, and *A. l. sahariensis*), were examined using Parsimony, Maximum Likelihood, and Bayesian Inference methods. Only two groups were identified, with individuals of *A. l. angusi*, *A. l. blainei*, *A. l. fassini*, *A. l. ornata*, and *A. l. sahariensis* forming one monophyletic clade (I) and samples of *A. l. lervia* forming a second clade (II). Low levels of genetic divergence (0.16%) among members of clade I indicated little differentiation among the five currently recognized subspecies; whereas levels of divergence (5.15%) between those subspecies (clade I) and *A. l. lervia* (clade II) approached levels typically observed between other species of bovids. Although statistically unsupported, members of clade II (*A. l. lervia*) formed two groups, with individuals from northwestern Algeria and northern Tunisia in one group and samples from northwestern Algeria and southern Morocco in a second group. These groups differed by 0.70%, indicating some level of populational differentiation. Together, these data bring into question the recognition of six subspecies of *Ammotragus lervia* and argue for a re-evaluation of aoudad taxonomy. Based on the available data, it appears prudent to recognize four subspecies of *A. lervia* (*blainei*, *lervia*, *ornata*, and *spp. novum*) and to subsume *angusi*, *fassini*, and *sahariensis* into *A. l. ornata*. Further, if the population status of aoudad in their native northern Africa is elevated from vulnerable to threatened or endangered, the large non-native, free-ranging populations in Texas and captive individuals at Fossil Rim Wildlife Center, Glen Rose, Texas, may prove to be a valuable source for attempts at genetic rescue considering there are potentially four subspecific lineages of aoudad in Texas.

Key words: *Ammotragus*, aoudad, barbary sheep, California, cytochrome-*b*, New Mexico, northern Africa, systematics, taxonomy, Texas

Introduction

Aoudad (*Ammotragus lervia*), also referred to as Barbary sheep, are native to montane regions of northern Africa. Over the last 120 years, populations have been successfully introduced into Croatia,

Czech Republic, Mexico, Spain, and the United States. Aoudad are listed as 'vulnerable' in their native range of northern Africa (Fig. 1) by the IUCN Red List of Threatened Species (Cassinello et al. 2022). However, introduced populations of aoudad in Texas have exponentially increased in size since the 1950 releases in the Post Oak Savannah (note that aoudad presently do not occur in this ecoregion) and Edwards Plateau ecoregions (Gould 1962; Mungall and Sheffield 1994) and their initial release in Palo Duro Canyon in 1957 (DeArment 1971; Mungall and Sheffield 1994). Dispersal of escapees from a New Mexico ranch into West Texas plus additional releases by private landowners (Simpson and Krysl 1981; Mungall and Sheffield 1994) in the Panhandle (i.e., High and Rolling Plains), Trans-Pecos, and Edwards Plateau regions of Texas (Gould 1962) further have propagated populations in new areas. In present day, aoudad populations in Texas are self-sustaining, increasing, and problematic, with more than 30,000 individuals occurring statewide (Traweek and Welk 1992; Wright et al. 2022).

Previous genetic studies (Derouiche et al. 2020; Stipoljev et al. 2021; Wright et al. 2022; Pizzigalli et al. 2023; Wright et al. in revision) used mitochondrial and nuclear markers to identify genetic relationships of aoudad as they pertain to the six recognized subspecies (*A. l. angusi*, *A. l. blainei*, *A. l. fassini*, *A. l. lervia*, *A. l. ornata*, and *A. l. sahariensis*) whose taxonomy was based on morphology and distribution (Allen 1939; Harper 1945; Ellerman and Morrison-Scott 1951; Ansell 1971; Gray and Simpson 1980; Cassinello 1998; Grubb 2005). Using cytochrome-*b* (*Cyt*b) datasets, Derouiche et al. (2020) and Pizzigalli et al. (2023) examined wild and semi-captive individuals across northern Africa. Derouiche et al. (2020) reported the occurrence of two subspecies (*A. l. lervia* and *A. l. sahariensis*), coinciding with the current distribution of the two subspecies in Algeria. Four nuclear markers also were examined but did not reflect the mitochondrial divergence between Mediterranean and Saharan populations and instead indicated the presence of a common genotype across Algeria (Derouiche et al. 2020). A more recent study by Pizzigalli et al. (2023) addressed the validity of five of the six subspecies (excluded *A. l. blainei*) in their native northern Africa and agreed with the findings of Derouiche et al. (2020); additionally, they identified a third mitochondrial lineage (subdivision within *A. l. lervia*) restricted to Tunisia.

Other studies (Stipoljev et al. 2021; Wright et al. 2022; Wright et al. in revision) focused on introduced populations in Europe and the United States, respectively. Stipoljev et al. (2021) used displacement loop (D-loop) and microsatellite loci to recognize four distinct haplotypes and three or four genetic clusters in non-native populations in Croatia, Czech Republic, and Spain. Wright et al. (2022) conducted a genetic assessment based on *Cyt*b and D-loop datasets from 232 non-native aoudad from portions of the American Southwest (California, New Mexico, and Texas), in combination with data presented in Derouiche et al. (2020) and Stipoljev et al. (2021). In the study by Wright et al. (2022), three mitochondrial lineages, two of which were similar to the subspecies *A. l. sahariensis*/*blainei* and *A. l. lervia*, were detected, indicating multiple introductions of the three respective aoudad subspecies into the US over a 90-year period. A third lineage could not be assigned to an existing subspecific name, due to a lack of samples from northern Africa, and therefore was treated as an unknown subspecies.

Data from nuclear loci have provided varying levels of resolution relative to the subspecific status of aoudad. Wright et al. (2022) reported low levels of genetic variation in exon 3 of the prion protein gene in aoudad and consequently could not contribute to the taxonomic knowledge of aoudad in the US. Pizzigalli et al. (2023) used microsatellites that identified the presence of five genetic clusters referred to as Egypt, Central Sahara (southeastern Algeria, Niger, and Egypt), Atlantic Sahara (Atlantic Sahara and southern Morocco), Tunisia, and Spain (Sierra Maria-Los Velez). Most recently, Wright et al. (in revision) used a range of 4,338 to 5,529 single nucleotide polymorphisms obtained from 73 individuals that potentially represented *A. l. blainei*, *A. l. lervia*, and *A. l. sahariensis* to address the subspecies issue. Principal components, isolation by distance, and Estimation of Effective Migration Surfaces analyses identified that mtDNA haplogroups and nuclear genotypes among the three examined subspecies were discordant, indicating that individuals from Fossil Rim Wildlife Center (FRWC), Glen Rose, Texas (*A. l. blainei*) were unique from all other populations of aoudad in Texas (*A. l. lervia* and *A. l. sahariensis*).

Unfortunately, Pizzigalli et al. (2023) was not able to obtain samples of *A. l. blainei* from native populations in Sudan or from captive sources at zoological

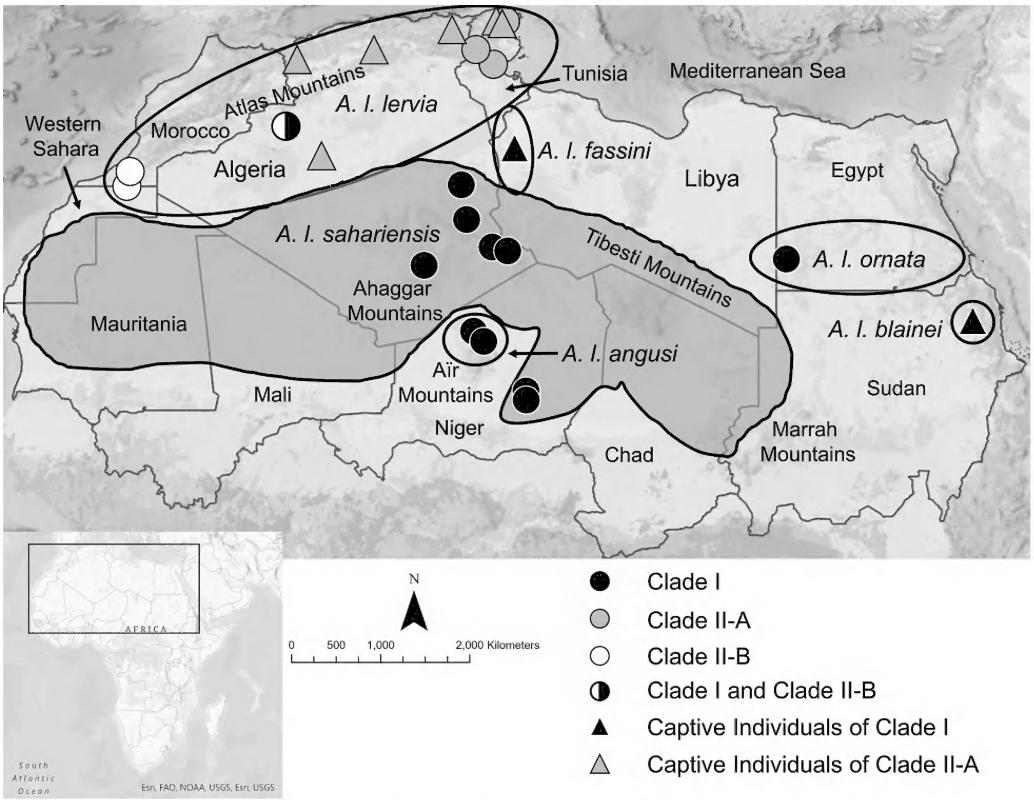

Figure 1. Map of northern Africa, with detailed topography to illustrate the geographical features in the native range of aoudad (*Ammotragus lervia*). Sampling locations of wild and captive aoudad and proposed subspecific distribution of *A. lervia* from Derouiche et al. (2020), Wright et al. (2022), and Pizzigalli et al. (2023) were superimposed with clade designations based on the cytochrome-*b* dataset herein.

operations. According to studbook records, individuals from Fossil Rim Wildlife Center are descended from *A. l. blainei* (Wright et al. 2022). Further, genetic differentiation between *A. l. blainei* and the other subspecies was apparent in both mitochondrial (Wright et al. 2022) and nuclear datasets (Wright et al. in revision), warranting a more detailed investigation. Herein, we combined the datasets of African aoudad from Derouiche et al. (2020) and Pizzigalli et al. (2023) and the dataset of aoudad primarily from Texas as well as California and New Mexico (Wright et al. 2022) to further decipher the mitochondrial lineages of aoudad in the United States. Further, we interpret those results in light of the genomic dataset revealed in Wright et al. (in revision).

METHODS AND MATERIALS

Sampling

Sampling efforts are summarized in Wright et al. (2022). An additional seven individuals were included from localities previously unrepresented from the extreme southern range of the Davis Mountains and the northern extension of the Sierra del Carmen through hunter-harvests facilitated by private landowners (see Appendix). Total samples ($n = 239$) from the southwestern United States include: 3 from California, 6 from New Mexico, and 230 from Texas. All tissue samples and museum specimens were deposited into the Robert J. Baker Genetic Resources Collection and Mammal Collection, respectively, of the Natural Science Research Laboratory at the Museum of Texas Tech University. Specimens collected from the above sampling efforts followed methods outlined in the guidelines of the American Society of Mammalogists (Sikes et al. 2016) and protocols approved by the Texas Tech University Animal Care and Use Committee (protocols #17023-02 and 20002-01).

Additional sequence data for *Cyt*b were obtained from NCBI GenBank and included samples examined in Derouiche et al. (2020), Wright et al. (2022), and Pizzigalli et al. (2023). Sequences from these studies were critical to determine the mitochondrial relationships as it pertains to subspecific designations from native northern Africa (Derouiche et al. 2020; Pizzigalli et al. 2023) compared to populations in the US (Wright et al. 2022).

DNA Sequencing

Genomic DNA (gDNA) was extracted from 0.1 g muscle using the Qiagen DNeasy blood and tissue extraction kit (Qiagen, Valencia, California). The entire *Cyt*b gene (1,143 bp) was amplified using the polymerase chain reaction (PCR) method (Saiki et al. 1988) with primers LGL765 (forward, Bickham et al. 1995) and LGL766 (reverse, Bickham et al. 2004), following the standard HotStarTaq (Qiagen Inc., Valencia, California) protocol: 25 µL reactions containing 3 µL gDNA, 12.5 µL HotStarTaq premix, 8.3 µL of double-distilled water, and 0.6 µL of each 10 µM primer. The thermal profile for PCR was as follows: hot start at 80°C, initial denaturation at 95°C for 2 min, followed by 34 cycles of denaturation at 95°C for 30 s, annealing at 45°C for 45 s, and extension at 73°C for 1 min, with a final extension at 73°C for 15 min.

PCR products were purified with ExoSAP-IT PCR Product Cleanup (Applied Biosystems, Foster City, California). Cycle sequencing reactions were conducted with BigDye Terminator v3.1 (Applied Biosystems, Foster City, California) using the following 1µM primers: LGL765 (Bickham et al. 1995), LGL766 (Bickham et al. 2004), and F1 (Whiting et al. 2003). Cycle sequencing products subsequently were purified using Sephadex filtration (Cytiva, Marlborough, Massachusetts) and centrifugation methods, followed by dehydration. Purified sequencing products were analyzed on an ABI 3730xl automated sequencer (Eurofins Genomics LLC, Louisville, Kentucky). Raw sequence reads were proofed using Sequencher 4.10.1 software (Gene Codes Corporation, Ann Arbor, Michigan) and associated chromatograms were visually examined to authenticate all base changes. All *Cyt*b sequences obtained in this study were deposited in GenBank (accession numbers: OR767276–OR767282).

Data Analyses

Phylogenetic analyses.—A parsimony analysis (PAUP* Version 4.0a169, Swofford 2003) was conducted on 281 (7 sampled herein and 274 acquired from GenBank) individuals from the *Cyt*b dataset with *Budorcas taxicolor* (Tibetan Takin, AY397661) and *Hemitragus jayakari* (Arabian Tahr, NC020621) as the outgroup taxa, based on Ropiquet and Hassanin (2005), to identify haplogroups and synapomorphies indicative of taxonomic identifications and assign individuals to a clade. It is important to note that sequences obtained from Derouiche et al. (2020) and Pizzigalli et al. (2023) were not complete sequences (i.e., <1,140 bp) for the *Cyt*b gene. However, datasets were not partitioned into various lengths. If sequences were missing data, then question marks were used to represent missing data for all analyses. Parsimony characters were assigned equal weight and variable nucleotide positions were treated as unordered, discrete characters with four possible states: A, C, G, and T, using the program PAUP* (Version 4.0a169, Swofford 2003). Phylogenetically uninformative characters were removed from the analy-

sis. The most-parsimonious trees were estimated using the heuristic search and tree-bisection-reconnection option. A strict consensus tree was generated from the population of most-parsimonious trees and a subsequent bootstrap analysis (Felsenstein 1985) with 1,000 iterations and the "fast" step-wise option was selected to evaluate nodal support, which we recognize as monophyletic clades.

Eighty-eight maximum likelihood (ML) models were evaluated using jModelTest-2.1.10 (Guindon and Gascuel 2003; Darriba et al. 2012). The Akaike information criterion with a correction for finite sample sizes (AICc, Hurvich and Tsai 1989; Burnham and Anderson 2004) identified the Hasegawa-Kishino-Yano model of nucleotide substitution (HKY, Hasegawa et al. 1985) and proportion of invariable sites model (HKY+I, -lnL = 2274.8399) as the most appropriate for the *Cyt*b dataset. However, the general time reversible (Tavaré 1986) plus proportion of invariable sites plus gamma distribution (GTR+I+Γ) model of nucleotide substitution, the most complex model, has been suggested to fit real data better than simpler models (Jayaswal et al. 2011; Sumner et al. 2012; Arenas 2015). We attempted several test runs using both models of HKY+I and GTR+I+Γ and determined that topologies and support did not differ. Therefore, we proceeded with the GTR+I+Γ model for all analyses. A likelihood analysis was performed using RAxML (Version 8.2.12, Stamatakis 2014) and the following parameters: base frequencies (A = 0.3226, C = 0.3003, G = 0.1255, and T = 0.2516), and the GTR+I+Γ model. Nodal support was evaluated using the bootstrap method (1,000 iterations, Felsenstein 1985), with bootstrap values (BS) ≥ 65 used to indicate moderate-to-strong nodal support.

A ML analysis under a Bayesian inference (BI) model (MrBayes v3.2.6, Ronquist et al. 2012) was conducted to generate posterior probability values (PPV). The GTR+I+Γ nucleotide substitution model and the following parameters were used: two independent runs with four Markov-chains (one cold and three heated; MCMCMC), 10 million generations, and sample frequency of every 1,000 generations from the last nine million generated. A visual inspection of likelihood scores resulted in the first 1,000,000 trees being discarded (10% burn-in) and a consensus tree (50% majority rule) constructed from the remaining trees. PPV ≥ 0.95 were used to designate nodal support (Huelsenbeck et al. 2002).

Genetic divergence.—Genetic distance values were estimated for selected clades using the Kimura 2-parameter model of evolution (Kimura 1980) using MEGA 11 (Tamura et al. 2021). The resulting values were used as levels of mitochondrial sequence divergence pertaining to the genetic species concept outlined in Bradley and Baker (2001) and Baker and Bradley (2006).

RESULTS

Phylogenetic analyses.— The three phylogenetic analyses (parsimony, ML, and BI) generated similar topologies in the *Cyt*b dataset. Consequently, each analysis is discussed in detail below; however, only the topology obtained from the BI analysis is shown (Fig. 2). Although there was genetic variation among individuals in terminal nodes, these associations were collapsed due to lack of nodal support, and consequently were represented as single unresolved groups (Clade I, Clade IIA, and Clade IIB).

For the parsimony analysis, the heuristic search was aborted due to computational limitations. A total of 43,619,010 rearrangements were tried before the analysis was terminated. Score of best tree found was 142 and number of trees retained was 223,238. A majority rule consensus tree was generated (not shown) that was similar in topology to the tree obtained in the BI analysis (Fig. 2); consequently, the bootstrap support values from the parsimony analysis were superimposed onto the BI topology. There was moderate to high bootstrap support for Clades I and II (BS = 99–100) as well as Subclade II-B (BS = 69). Fifty-seven nucleotide and seven codon substitutions (T60M, L121F, T122A, M240T, I303L, M309T, and I348M) were phylogenetically informative between Clades I and II and were superimposed onto the topology obtained from the BI analysis, with 25 nucleotide substitutions acting as synapomorphies for Clade I and 32 nucleotide and seven codon substitutions acting as

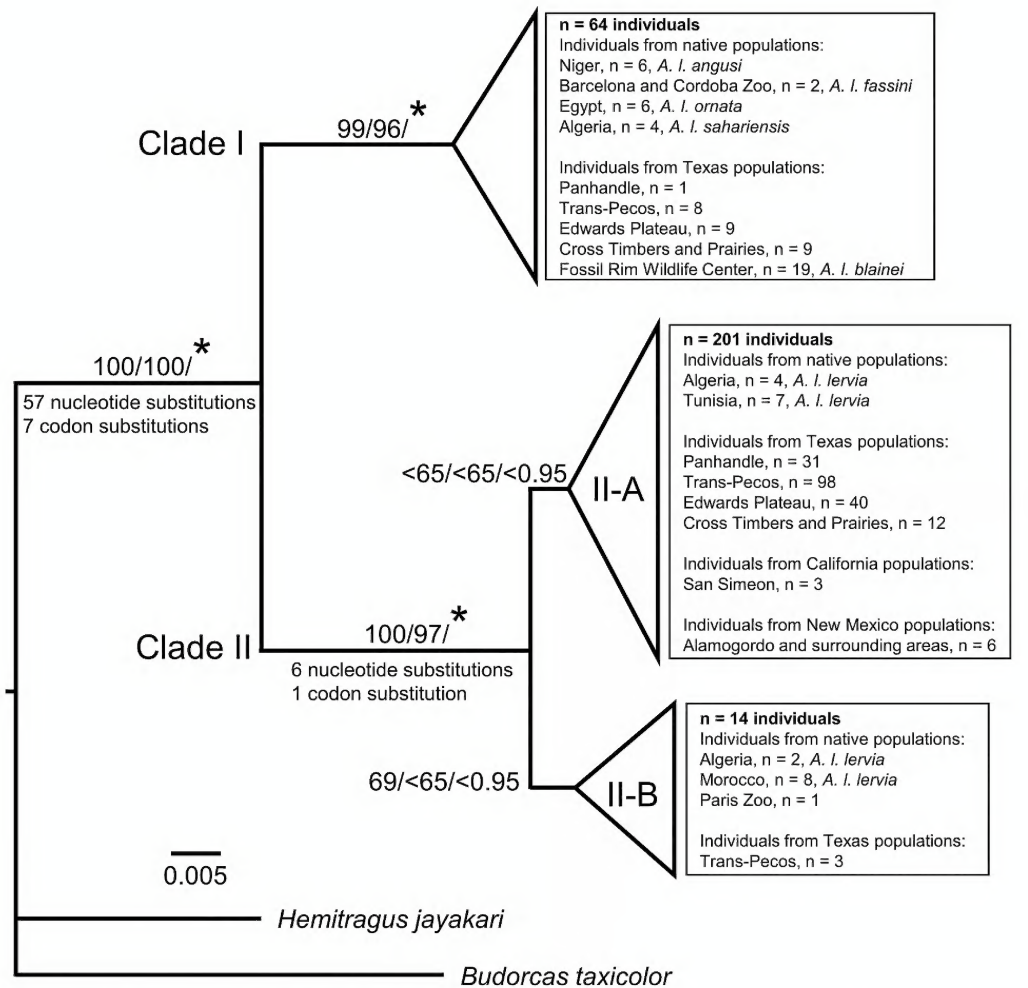
n = 64 individuals
Individuals from native populations:
Niger, n = 6, A. l. angusi
Barcelona and Cordoba Zoo, n = 2, A. l. fassini
Egypt, n = 6, A. l. ornata
Algeria, n = 4, A. l. sahariensis
Individuals from Texas populations:
Panhandle, n = 1
Trans-Pecos, n = 8
Edwards Plateau, n = 9
Cross Timbers and Prairies, n = 9
Fossil Rim Wildlife Center, n = 19, A. l. blainei
Clade I
99/96/*
100/100/*
57 nucleotide substitutions
7 codon substitutions
<65/<65/<0.95
II-A
n = 201 individuals
Individuals from native populations:
Algeria, n = 4, A. l. lervia
Tunisia, n = 7, A. l. lervia
Individuals from Texas populations:
Panhandle, n = 31
Trans-Pecos, n = 98
Edwards Plateau, n = 40
Cross Timbers and Prairies, n = 12
Individuals from California populations:
San Simeon, n = 3
Individuals from New Mexico populations:
Alamogordo and surrounding areas, n = 6
Clade II
100/97/*
6 nucleotide substitutions
1 codon substitution
69/<65/<0.95
II-B
n = 14 individuals
Individuals from native populations:
Algeria, n = 2, A. l. lervia
Morocco, n = 8, A. l. lervia
Paris Zoo, n = 1
Individuals from Texas populations:
Trans-Pecos, n = 3
0.005
Hemitragus jayakari
Budorcas taxicolor

Figure 2. Phylogeny of the cytochrome-*b* gene using 279 individuals of aoudad (*Ammotragus lervia*). Parsimony (left of the first slash) and Maximum Likelihood (in the middle of the two slashes) bootstrap values ≥ 65 indicate nodal support. Bayesian posterior probability values are indicated by the * and represent ≥ 0.95 nodal support (right of the slash).

synapomorphies for Clade II. Further, six nucleotide and one codon (T190A) substitution differentiated Subclades II-A and II-B.

In the BI analysis, two supported clades were identified (I and II). Clade I was comprised of a total of 64 individuals from Niger (*A. l. angusi*), Barcelona and Cordoba Zoos (*A. l. fassini*), Egypt (*A. l. ornata*), and Algeria (*A. l. sahariensis*) as well as Texas populations from several ecoregions (Panhandle, Trans-Pecos, Edwards Plateau, and Cross Timbers and Prairies) and Fossil Rim Wildlife Center (*A. l. blainei*). Clade II was separated into two subclades (A and B) and thus represent unresolved polytomies. Subclade II-A contained

a total of 201 individuals from Algeria (*A. l. lervia*) and Tunisia (*A. l. lervia*) as well as individuals from California and New Mexico and several ecoregions in Texas (Panhandle, Trans-Pecos, Edwards Plateau, and Cross Timbers and Prairies). Subclade II-B was supported by Parsimony and BI analyses and comprised of 14 individuals from Algeria (*A. l. lervia*), Morocco (*A. l. lervia*), and the Paris Zoo as well as individuals from the Trans-Pecos ecoregion of Texas. The ML analysis also produced a topology (not shown) that was nearly identical to the topology obtained from the BI analysis. Bootstrap support values obtained from the ML analysis were superimposed onto the BI topology (Fig. 2).

Genetic divergence.— Estimation of the Kimura 2-parameter (Kimura 1980) using the *Cyt*b dataset indicated an overall mean genetic distance of 1.90% for all aoudad (n = 279) included in the study. The genetic distances within selected clades were as follows: clade I = 0.16%; clade II = 0.11%; clade II-A = 0.02%; and clade II-B = 0.18%. Estimates of genetic distances between selected clades were: 5.15% between clade I and clade II; 5.16% between clade I and clade II-A; 5.08% between clade I and clade II-B; and 0.70% between clade II-A and clade II-B.

DISCUSSION

DNA sequence data from the mitochondrial *Cyt*b gene were obtained from all six recognized subspecies of *A. lervia*. Although mitochondrial markers identified unique haplotypes in both native and introduced populations (Derouiche et al. 2020; Stipoljev et al. 2021; Wright et al. 2022; Pizzigalli et al. 2023), there were not clear haplotype delineations that could be associated with subspecific designations. For example, Clade I contained individuals representing *A. l. angusi*, *A. l. fassini*, *A. l. ornata*, and *A. l. sahariensis* from native populations in northern Africa, introduced populations in Texas (Panhandle, Trans-Pecos, Edwards Plateau, and Cross Timbers and Prairies), and captive descendants of *A. l. blainei* and *A. l. fassini* from FRWC and the Barcelona and Cordoba Zoos, respectively (Fig. 2). These five subspecies were genetically similar and presumably could be collapsed into a single taxon. However, genetic clustering and phylogeographic structure was observed using microsatellite (Stipoljev et al. 2021; Pizzigalli et al. 2023) and RAD-seq datasets (Wright et al. in revision) producing a discordancy with the mitochondrial and nuclear lineages of aoudad. For the sixth subspecies, *A. l. lervia*, there appears to be a division in mitochondrial lineages among populations in Algeria, Morocco, and Tunisia (Pizzigalli et al. 2023; Fig. 1, Fig. 2). For example, Clade II-A is comprised of individuals from Algeria and Tunisia representing *A. l. lervia* as well as populations in California, New Mexico, and Texas whereas Clade II-B contains individuals from Algeria and Morocco representing *A. l. lervia* as well as populations in the Trans-Pecos region of Texas (Fig. 2).

Based on mtDNA and microsatellites, Pizzigalli et al. (2023) suggested that *A. l. angusi*, *A. l. fassini*, *A. l. ornata*, and *A. l. sahariensis* be considered a single subspecies, with the caveat that *A. l. ornata* may prove to be a separate subspecies, and proposed a distribution extending from northwestern Algeria to Egypt. Pizzigalli et al. (2023) further suggested that populations in northwestern Algeria and Morocco (i.e., Atlantic Sahara) be considered a unique taxonomic unit (i.e., a new subspecies) with a distribution ranging from southern Morocco to the province of Béchar, Algeria. Finally, Pizzigalli et al. (2023) recommended that *A. l. lervia* be retained as a distinct subspecies whose distribution is restricted to Tunisia.

For the most part, our data are in agreement with Pizzigalli et al. (2023). First, we concur that *A. l. angusi*, *A. l. fassini*, *A. l. ornata*, and *A. l. sahariensis* should be considered as a single subspecies based on the low level of genetic divergence detected among these subspecies (0.16%) as outlined in Baker and Bradley (2006). If this scenario proves to be correct, *A. l. ornata* would have nomenclatorial priority (Geoffry-Saint Hilaire 1827). Second, the recognition of *A. l. ornata* as a separate subspecies is not supported based on the mtDNA dataset presented herein; however, more robust nuclear and morphological analyses may prove

otherwise (see Pizzigalli et al. 2023). Third, although Pizzigalli et al. (2023) could not comment on the status of *A. l. blainei* (no samples available to them), in Wright et al. (2022), Wright et al. (in revision), and the current study, *A. l. blainei* represented a distinct genetic clade based on extensive mtDNA and RAD-seq datasets. Consequently, it appears that *A. l. blainei* should be recognized as a separate subspecies until further data become available. Fourth, levels of divergence (5.15%) between subspecies contained in clade I (*A. l. angusi*, *A. l. blainei*, *A. l. fassini*, *A. l. ornata*, and *A. l. sahariensis*) and clade II (*A. l. lervia*) approached levels of genetic variation typically observed between other species of bovids (Wright et al. 2022). Fifth, although unsupported by posterior probability values (Fig. 2), members of the *A. l. lervia* clade (clade II) formed two groups, with samples from northwestern Algeria and northern Tunisia in one group (clade II-A) and samples from northwestern Algeria and southern Morocco forming a second (clade II-B). These groups (clade II-A and clade II-B) differed by 0.70%, indicating some level of populational differentiation. We agree with Pizzigalli et al. (2023) that the populations near northwestern Algeria and northern Tunisia may represent *A. l. lervia* and the populations near northwestern Algeria and Morocco may represent an unknown subspecies.

There is no clear geographical landmass, barrier, or other feature (Fig. 1) that may have caused the mitochondrial division within *A. l. lervia* that occurred approximately 1.25 million years ago (Wright et al. 2022). Northern Africa, which spans the native range of aoudad, has experienced dramatic changes in rainfall over long periods of time and historical climatic changes may have influenced the genetic structuring of aoudad populations as well. It may be that more in-depth nuclear analyses (e.g., next generation sequencing technologies) are required to tease apart phylogeographic relationships. Consequently, the third lineage that could not be assigned to an existing subspecific taxon in Wright et al. (2022) is represented by Clade II-B (Fig. 2) and most likely is an unknown subspecies, but it is more closely related to *A. l. lervia* than any of the other five possible subspecies. Perhaps, portions of the Atlantic Sahara to Egypt were all connected at one time and potentially represented a single lineage (*A. l. ornata*) with the other three subspecies (*A. l. angusi*, *A. l. fassini*, and *A. l. sahariensis*) being synonymous with *A. l. ornata*.

Considering that potentially five of the six subspecies of aoudad (*A. l. angusi*, *A. l. blainei*, *A. l. fassini*, *A. l. ornata*, and *A. l. sahariensis*) possessed similar mitochondrial haplotypes and that one of the six subspecies of aoudad (*A. l. lervia*) demonstrated a division in their mitochondrial haplotypes, caused by an unknown phylogeographic factor, more samples range-wide from northern Africa and novel sequencing techniques (whole genome sequencing, exome data, shotgun sequencing, restriction-site associated DNA sequencing, etc.) are needed to further identify the validity of these taxonomic subspecies. A morphometric study on native and non-native populations of aoudad is needed to further delineate between subspecies in conjunction with genetic and genomic datasets and to address the conservation status of aoudad in northern Africa.

With the current mitochondrial dataset, presented herein, of captive and non-native, introduced aoudad populations in California, New Mexico, and Texas combined with representative sequences of aoudad from their native range of northern Africa, it appears that there are potentially four lineages of aoudad (*A. l. blainei*, *A. l. lervia*, *A. l. ornata*, and *spp. novum*, where *A. l. angusi*, *A. l. fassini*, and *A. l. sahariensis* are subsumed into *A. l. ornata*) in the southwestern US. Although the paucity of translocation and introduction records limits our interpretation of aoudad taxonomy and genetic diversity, it is clear that all clades (I, II-A, and II-B) are represented on the landscape based on the mitochondrial dataset herein. Importantly, individuals of *A. l. blainei* are maintained in captivity at FRWC. The combination of large non-native, free-ranging populations in Texas and captive individuals at FRWC could prove to be a valuable source for attempts at genetic rescue (e.g., reintroduction, rewilding, etc.) if the population status of aoudad in their native northern Africa elevates from vulnerable to threatened or endangered.

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APPENDIX

For each specimen examined, localities associated with a TK number (special number of the Museum of Texas Tech University) and NCBI GenBank accession number (cytochrome-b) are provided in parentheses and are separated by slashes.

Ammotragus lervia.—Texas: Brewster County, Sierra Del Carmen, El Carmen Land and Conservation Company (CEMEX USA) (TK260167/OR767276; TK260168/OR767277; TK260169/OR767278); Jeff Davis County, Davis Mountains, Calamity Creek, 30.516583, -103.827083 (TK260170/OR767279); 30.52139, -103.82617 (TK260171/OR767280; TK260172/OR767281; TK260173/OR767282).

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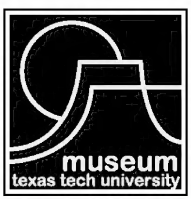

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